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Nitrogen isotope constraint on the zonation of multiple transformations between dissolved and particulate organic nitrogen in the Changjiang plume



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HIGHLIGHTS

- DON deficit and negative $\delta^{15} N_{\text{DON}}$ shift reveals DON's nonconservative behavior.
- Nonconservative DON behavior is driven by different N processes in river plume.
- DON assimilation contributes ~16% of the PN production in offshore surface plume.
- Active transformations had occurred between the DON and PN pools.

GRAPHICAL ABSTRACT



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ABSTRACT

Information on the sources and transformations of particulate organic N (PN) and dissolved organic N (DON) at the coastal interface remains insufficient due to technological difficulties and complicated features of intensive physical mixing and rapid biological activities. Here, we investigated the spatial distribution of concentrations and isotopic compositions of PN and DON in the Changjiang plume during the summer flood period. In average, DON and PN accounted for $25.6 \pm 12.1\%$ and $8.1 \pm 9.1\%$ (n = 55), respectively, of the total N pool, with the remaining N primarily in the form of nitrate (NO₃⁻). Mean δ^{15} N values were the lowest for DON ($-0.1 \pm 2.7\%$, n = 58) and slightly higher for PN ($2.0 \pm 1.6\%$, n =101), and the highest for NO₃⁻ (6.5 \pm 2.2‰, n = 67), suggesting multiple transformations had occurred to differentiate isotopic characteristics among the three N pools. By applying a conservative mixing model, we found DON deficits (-3.5 \pm 3.7 µmol L⁻¹, n = 43) and negative shift in $\delta^{15}N_{DON}$ (-3.6 \pm 2.2‰, n = 43) in the Changjiang plume, revealing nonconservative DON behaviors. In the offshore surface plume where Chlorophyll a was high, the most likely cause is the DON uptake by phytoplankton with a strong inverse isotope effect (around -40%). This DON assimilation by phytoplankton contributed to ~16 \pm 12% of the PN production, with the remaining supported by NO_3^- assimilation, producing an overall isotope effect of 4–9%. However, in waters near the river mouth and at the bottom of the offshore plume where total suspended matter concentrations were high ($>5 \text{ mg L}^{-1}$), the DON deficit was most likely induced by the selective adsorption of ¹⁵N enriched moieties of DON onto particulate surfaces

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(with an isotope effect of -20% to -5%). Unlike dissolved organic carbon to behave conservatively in most estuaries, our results show that active transformations had occurred between the DON and PN pools in the Changjiang plume.

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1. Introduction

Nitrogen (N) is a vital element for living organisms and generally limits marine productivity. Over the past century, N input from rivers into estuaries and coastal oceans have been substantially increased. This N input not only leads to negative environmental consequences (i.e., eutrophication, seasonal hypoxia), but also impacts the biogeochemical cycles of multiple elements (i.e., C, N, P) at the land-ocean boundary. The Changjiang (also known as the Yangtze River), the sixth largest river globally, discharges huge amounts of freshwater $(30,166 \text{ m}^3 \text{ s}^{-1} \text{ on average})$ and reactive N, including particulate/dissolved organic and inorganic N species, into the East China Sea, particularly during the wet summer season (Gao et al., 2012; Zhang et al., 2007). The freshwater extends offshore in the summer, known as the Changjiang plume, and is characterized by high nutrient concentrations (dissolved inorganic nitrogen [DIN]: 1.7–160 µmol L⁻¹, dissolved inorganic phosphorus [DIP]: 0.1–2.6 μ mol L⁻¹, respectively), as well as high nitrogen to phosphorus (N/P) ratios (16-200) and rates of biological activities (Tseng et al., 2014). As riverine nutrient loadings, N in particular, have increased in recent decades, occurrences of harmful algal blooms and seasonal hypoxia of bottom waters in the region of the Changjiang plume have also increased significantly (Li et al., 2014; Wang et al., 2017; Zhu et al., 2011). Investigating changes in particulate organic N (PN) and dissolved organic N (DON) concentrations and isotopic compositions spatially throughout the plume will benefit our understanding of the N cycle, revealing the influence of diverse N sources and transformation processes, and potentially identifying mitigation strategies for anthropogenic eutrophication in river-dominated coastal environments.

Three major N forms, nitrate (NO₃⁻), DON, and PN coexist in the Changjiang plume. Similar to other large rivers (Möbius and Dähnke, 2015; Pennino et al., 2016; Wells and Eyre, 2018), NO₃⁻ dynamics have been relatively well-studied and recognized in the Changjiang plume (Gao et al., 2015; Liu et al., 2016a; Yan et al., 2017). However, the distribution of DON remains unexplored in this area despite its long-recognized role in supporting both bacteria (Andrews and Williams, 1971; Calleja et al., 2013) and phytoplankton production (Bronk et al., 2007; Glibert et al., 2004; Moschonas et al., 2017). A recent study in the southern ECS shelf indicated that DON may stimulate dinoflagellate blooms (Zhang et al., 2015), underscoring the significance of DON in contributing to harmful algal blooms. However, due to technological difficulties in measuring DON isotopic compositions ($\delta^{15}N_{DON}$), our understanding of N cycle in the Changjiang plume remains insufficient.

Several studies have been carried out to identify the autochthonous and allochthonous sources of PN in the Changjiang estuary and adjacent coastal areas by measuring its stable isotopic composition ($\delta^{15}N_{PN}$) (Gao et al., 2014; Liu et al., 2018; Wu et al., 2003; Zhang et al., 2007). Three main sources of PN, including terrestrial inputs, marine sources and deltaic sediments, have been reported that could contribute to the PN pool in the water column of the Changjiang plume area (Gao et al., 2008; Kao et al., 2003; Zhang et al., 2007). However, PN is not conservative due to continuous biological alterations during its transport (Wu et al., 2016; Yang et al., 2017; Zhang et al., 2007). Thus, $\delta^{15}N_{PN}$ may reflect a combination of its original signature (different PN sources with diverse $\delta^{15}N$ values) and subsequent biological transformations (decomposition and formation), such as observed in $\delta^{15}N$ signals during NO₃⁻ assimilation (Tamše et al., 2014) and/or DON utilization (Sigman and Fripiat, 2018; Sipler and Bronk, 2015). Nonetheless, simultaneous measurements of PN, DON and NO_3^- concentrations and isotopic compositions in the Changjiang plume have not yet been documented, and may provide insightful information into the cycling of major N pools.

In this study, we investigated the spatial distribution of the concentrations and isotopic signatures of multiple N species in the Changjiang plume during summer 2011 to reveal the potential sources and processing of the DON and PN pools. Comparing relative changes in the amount and isotopic composition of N in DON, PN and NO₃⁻⁻ will help us to understand N dynamics in this river-influenced coastal area, and the results may have strong implications for other similar river-dominated ocean margins.

2. Materials and methods

2.1. Study area and water sampling

Sampling and observations of the Changjiang plume were performed onboard the R/V *Runjiang I* during August 2011. Water samples for chemical analyses were collected at a total of 26 stations from the river mouth moving offshore, covering areas significantly influenced by the Changjiang plume (Fig. 1), using a rosette sampler fitted with 12 L Niskin bottles. The sampling depth interval was modified based on the bottom depth of each station; at stations close to the river mouth (from YOA to YOE), only one sample was taken near the surface layer (~3 m). At other stations, 2 to 5 samples were collected from ~3 m to the near-bottom layer. Vertical profiles of temperature and salinity were recorded by a CTD profiler (SBE-911 plus, Sea-Bird Co.) at a sampling frequency of 3 Hz, and data were reported as 1 m interval averages.

2.2. Analysis of PN

Water samples for the measurements of concentrations and isotopic compositions of PN were filtered (0.5–4.0 L) through Whatman GF/F membranes (47 mm diameter, 0.7 µm pore size, pre-combusted at 450 °C for 4 h) under mild vacuum. The filters containing the particulate material were preserved at -20 °C until analysis. Prior to analysis approximately 1 mL of 1 mol L^{-1} HCl (ACS-grade, 37%) was added directly to the filters to remove carbonates. Such acid treatment has been widely used and verified that its influence on the measurement of PN and $\delta^{15}N_{PN}$ is negligible (Casciotti et al., 2008; Kao et al., 2012). Filters were then dried at 60 $^\circ C$ for 48 h, transferred to tin capsules and compressed into small packages. They were then analyzed for PN and $\delta^{15}N_{PN}$ with an Elemental Analyzer interfaced with an Isotope Ratio Mass Spectrometer (EA-IRMS) operating in continuous-flow mode at the Key Laboratory of Marine Chemistry Theory and Technology, Ocean University of China. We use delta notation to describe the N isotopic ratios ($\delta^{15}N/\infty$), where $\delta^{15}N = [({}^{15}N/{}^{14}N)_{sample}/({}^{15}N/{}^{14}N)_{standard} -$ 1]. Here, $({}^{15}N/{}^{14}N)_{sample}$ and $({}^{15}N/{}^{14}N)_{standard}$ are the ${}^{15}N/{}^{14}N$ ratio for sample and standard, respectively. N₂ in air is the N isotope reference.

A standard curve was made using an international reference standard (U.S. Geological Survey (USGS) 40, L-glutamic acid with a N% of 9.5%) to calibrate the N content. For the calibration of δ^{15} N, three international isotope standards (USGS 40: –4.5%; IAEA-600: 1.0%; USGS 41: 47.5%) were used here. To monitor the data quality, reference standard was analyzed at ten sample intervals. The analytical precision of δ^{15} N_{PN}



Fig. 1. Sampling locations in the Changjiang plume. The crosses and circles represent stations with measurements of PN and DON, respectively. The blue line indicates the plume transect starting from Y0A to Y5. The yellow shade is the boundary of Zone 1, while the violet shade represents Zone 2, of which zoning was applied to discuss the nonconservative behaviors of DON for convivence. Water samples for Zone 2 were mostly collected from the upper surface water (<20 m) with Chlorophyll *a* (Chl *a*) >2 μ g L⁻¹. Then the remaining water samples were belonged to Zone 3. Bathymetry is shown as blue contours.

measurements was better than $\pm 0.2\%$ based on the long-term reproducibility for reference standards and field samples (Kao et al., 2012; Yang et al., 2017).

2.3. Analysis of DON

Water samples for the measurement of DON and $\delta^{15}N_{DON}$ were filtered through cellulose acetate membrane filters (47 mm diameter, 0.45 μm pore size, acid-cleaned) and stored in acid-cleaned vials at $-20~^\circ C$ until analysis.

Samples were thawed at room temperature, and 10 mL of each was transferred to borosilicate glass test tubes (15 mL, pre-combusted at 450 °C for 4 h). Next, 2 mL of persulfate oxidizing reagent (POR, a mixture solution of recrystallized potassium persulfate and sodium hydroxide, both of two reagents were in the certified ACS-grade), which was prepared following the procedure described by Knapp et al. (2005), was added to each test tube. The Teflon-lined caps (acid-cleaned and dried at 60 °C for 12 h) were then tightly closed, and the samples were mixed and digested at 100 °C for 60 min in a thermostatic water bath. Meanwhile, three blanks (10 mL POR) and two different laboratory standards (Glycine and EDTA, ACS-grade) were also digested to determine the reagent blank and analyte recoveries, respectively. After digestion, the samples, blanks and standards were allowed to cool, and the pH was reduced down to ~6 (suitable for bacterial conversion during the measurement of $\delta^{15}N_{TDN}$ using the denitrifier method) by adding 6 mol L^{-1} HCl (ACS-grade, 37%). Then, total dissolved N (TDN, converted into NO₃) was measured by the chemiluminescence method, with a detection limit of 0.05 μ mol L⁻¹ (Knapp et al., 2005; Xu et al., 2017). [DON] was calculated by subtracting the $[NO_3 + NO_2]$ (the sum of the concentrations of NO_3^- and nitrite) values of water samples without persulfate treatment and the POR blank from the [TDN]. Noteworthy, DON in this study may include NH_{4}^{+} ; however, the influence of [NH₄⁺] on DON was limited since [NH₄⁺] contributed averagely 4.2 \pm 5.6% (n = 63) to DON. Its influence on $\delta^{15}N_{DON}$ was assessed by assuming $\delta^{15}N_{\text{NH4}}$ range of -5.0% to 5.0%, which results in an isotopic alteration of -1.1% to 1.1%. For the entire range of δ^{15} N_{DON} (-9.0% to 5.5%) observed in this study area, the isotope bias from [NH₄⁺] would not influence the offset calculation for DON and $\delta^{15}N_{DON}$ (see Section 4.2). Therefore, DON+NH⁺₄ is referred to as DON hereafter. The equations involved in this study are as follows:

$$[TDN] \times V = [DON] \times V_S + [NO_3 + NO_2] \times V_S + [POR] \times V_{POR}$$
(1)

 $[TDN] \times \delta^{15}N_{TDN} \times V = [DON] \times \delta^{15}N_{DON} \times V_S + [NO_3 + NO_2] \times \delta^{15}N_{(NO3+NO2)} \times V_S$

$$+[POR] \times \delta^{15} N_{POR} \times V_{POR} \tag{2}$$

$$[\text{DON}] = \frac{[\text{TDN}] \times \text{V} - [\text{NO}_3 + \text{NO}_2] \times \text{V}_S - [\text{POR}] \times \text{V}_{\text{POR}}}{\text{V}_S}$$
(3)

$$\delta^{15}N_{TDN} = \frac{[DON] \times \delta^{15}N_{DON} \times V_S + [NO_3 + NO_2] \times \delta^{15}N_{(NO3+NO2)} \times V_S + [POR] \times \delta^{15}N_{POR} \times V_{POR}}{[TDN] \times V}$$

$$\delta^{15}N_{DON} = \frac{[TDN] \times \delta^{15}N_{TDN} \times V - [NO_3 + NO_2] \times \delta^{15}N_{(NO3+NO2)} \times V_S - [POR] \times \delta^{15}N_{POR} \times V_{POR}}{[DON] \times V_S}$$
(5)

Here, [TDN] is the concentration of NO₃⁻ in a sample after oxidation, while [DON], [NO₃ + NO₂] and [POR] are the concentrations of DON, NO₃ + NO₂, and the POR blank, respectively. $\delta^{15}N_{TDN}$ is the $\delta^{15}N$ value of TDN, while $\delta^{15}N_{DON}, \delta^{15}N_{(NO3+NO2)},$ and $\delta^{15}N_{POR}$ are the $\delta^{15}N$ values of DON, NO₃ + NO₂ and the POR blank, respectively. V₅ and V_{POR} are the volumes of water samples and POR blanks, respectively, and V is the sum of V_S and V_{POR}. Note that the NO₃⁻ concentrations and isotopic compositions have previously been reported by Yan et al. (2017). Those reported data for NO₃⁻ are used here for comparison with the PN and DON pools and in-depth data interpretation in this study.

After the oxidation of TDN to NO_3^- , the NO_3^- in the samples, blanks and standards was microbially converted to nitrous oxide (N_2O) using the denitrifier method (Casciott et al., 2002; Sigman et al., 2001). Subsequently, N_2O was measured using a gas chromatograph (GasBench II) connected to an IRMS system (Thermo Scientific DELTA V advantage). Four international NO_3^- isotope standards (International Atomic Energy Agency-N3: 4.7‰, USGS 34: -1.8%, USGS 35: 2.7‰ and USGS 32: 180‰) and were used to calibrate the $\delta^{15}N$ (Böhlke



et al., 2003). Nitrate reference standard (13.7‰) was analyzed per ten samples for quality control. The analytical precision was better than $\pm 0.2\%$ for δ^{15} N using the denitrifier method in our laboratory (Xu et al., 2017; Yan et al., 2019; Yan et al., 2017).

The results of reagent blanks used for correcting measurements of all DON samples are summarized in Table S1, showing that the concentrations and isotopic compositions of POR blanks ranged from 1.4–4.8 μ mol L⁻¹ and -15.0% to -46.0%, respectively. Such variability may be attributed to the differences of purification efficiencies and reagent batches in each experiment. However, the true concentrations and isotopic compositions of DON in the samples can be obtained using the mass balance shown in Eqs. (1)-(5). The measured isotopic values of laboratory standards (1.2 \pm 0.1‰, n = 10 for Glycine and -0.6 ± 0.1 %, n = 9 for EDTA) were close to the modeled values calculating from Eq. (4), which considers the influence of POR (Fig. S1), suggesting that the oxidation efficiency near 100% by using POR. However, the deviations between the measured and true $\delta^{15}N$ values of standards indicated that lower TDN concentrations result in a greater influence from POR. After the POR correction, lab standards showed results close to the expected values (Fig. S1). Therefore, $\delta^{15}N_{DON}$ in a given sample can be obtained from Eqs. (4)–(5). The analytical precision was better than $\pm 0.3 \text{ }\mu\text{mol} \text{ }L^{-1}$ for DON and $\pm 0.5\%$ for $\delta^{15}N_{DON}$.

3. Results

3.1. Spatial distribution of parameters in the surface plume

The sea surface temperature was highest (>28 °C) near the river mouth (Fig. 2a), followed by the outermost stations (Y5 and Y5a, 27.4 °C). Two low-temperature centers were detected in the north (Y12 and Y16, 23.5–23.8 °C) and center (Y8, 22.7 °C) of the sampling area. Sea surface salinity increased seaward (Fig. 2b), with the lowest value in the river mouth (Y0A, 2.2). Two low-salinity tongues were observed extending northeast and southeast, respectively. Surface total suspended matter (TSM) showed a sharp eastward decreasing trend (Fig. 2c). Near the river mouth (YOA and YOB), TSM concentrations were >2000 mg L⁻¹. TSM decreased seaward to 5 mg L⁻¹ rapidly along the ~123°E transect, which has been previously defined as the turbidity front (red bold line in Fig. 2c), then steeply declined further to ~1 mg L^{-1} in the outer plume. Interestingly, Chl *a* measurements revealed three high-concentration patches (Fig. 2d); one at the river mouth (Y0B, 20.9 mg L^{-1}), and the other two (2Y11, 72.4 mg L^{-1} ; 2Y9a and Y9b, 48.0–52.1 mg L^{-1} just off and parallel to the turbidity front. A decreasing seaward trend was observed in NO₃⁻ concentrations (Fig. 2e), yet, values of $\delta^{15}N_{NO3}$ increased significantly offshore (Fig. 2f).

Similar to the TSM pattern, we observed a decreasing seaward trend in PN concentrations (Fig. 2g). At the river mouth, PN was as high as 140.8 µmol L⁻¹ (YOB); however, it generally remained at ~1 µmol L⁻¹ beyond the turbidity front in the outer plume. Contrary to PN, $\delta^{15}N_{PN}$ showed a much different spatial pattern (Fig. 2h); $\delta^{15}N_{PN}$ values were around 1‰ in the inner plume, decreasing to <-1‰ inside the high-Chl *a* patches off the turbidity front (Fig. 2d), and increasing eastward until reaching 4.1‰ at station Y6.

Generally, DON decreased moving off the coastline (Fig. 2i). DON was as high as 32.7 μ mol L⁻¹ at the river mouth, while concentrations remained ~8 μ mol L⁻¹ in the middle and outer plume. Similar to DON, $\delta^{15}N_{DON}$ also showed a decreasing trend offshore (Fig. 2j), except for

the lowest value (-3.2%) observed at 2Y9a. Values of $\delta^{15}N_{DON}$ declined from >4.0% near the river mouth to <1.0% in the north section of the outer Changjiang plume.

3.2. Vertical distribution of parameters along the Changjiang plume transect

We present vertical distribution of parameters along the Changjiang plume transect from station YOA to station Y5 (Fig. 3). Hightemperatures and low-salinities were observed near the river mouth (distance <40 km) where the water was vertically well-mixed (Fig. 3a and b). However, strong stratification occurred at water depths over 20 m. In this area of the plume, water temperature decreased monotonically as water depth increased, while salinity showed an opposite trend. A patch of low-temperature (<19.9 °C) and high-salinity (>34.3) water was observed in the bottom water of stations Y4 and Y4a, suggesting the influence of the Northern Kuroshio Branch Current (NKBC) as indicated by previous study (Yang et al., 2012). TSM was vertically well-mixed near the river mouth (Fig. 3c), but exhibited a strong vertical gradient at stations with water depths between 20 m and 40 m, increasing with depth in our profiles. In the offshore stations (>40 m) we observed less vertical variability but a similar pattern emerged: TSM kept concentrations remained $<5 \text{ mg L}^{-1}$ in the upper layer but were $>5 \text{ mg L}^{-1}$ in the deepest sample. Similar to water temperature, Chl a concentrations exhibited a well-mixed pattern in the river mouth, but a strong vertical gradient was observed in the offshore stations (Fig. 3d). Chl *a* decreased from 47.9–52.1 μ g L⁻¹ in the upper layer at stations Y9b and 2Y9a to $<1.0 \ \mu g \ L^{-1}$ in the lower layer. The NO₃⁻ concentrations decreased dramatically with increasing distance offshore (Fig. 3e), with a value of $>100 \,\mu\text{mol}\,\text{L}^{-1}$ near the river mouth to $<10 \mu mol L^{-1}$ at offshore stations. However, a distinct pattern was found in the vertical distribution of $\delta^{15}N_{NO3}$ (Fig. 3f), with highest values (>11.0%) in the offshore surface layer but lowest values (<5.0%) at the bottom waters.

Similar to TSM, concentrations of PN decreased significantly moving offshore along the plume transect (Fig. 3g). Vertically, PN showed a well-mixed pattern near the river mouth, while increasing with depth in the middle plume. However, in the outer plume, PN concentrations were mostly <1 µmol L⁻¹, with the exception of the surface and bottom layers. The vertical distribution of $\delta^{15}N_{PN}$ was relatively uniform (typically between 1.0‰ and 3.0‰) within 100 km offshore (Fig. 3h). Beyond this distance, an obvious vertical gradient in $\delta^{15}N_{PN}$ developed, with values increasing from <0‰ in the upper layer to >3.4‰ in the bottom layer of the outer plume.

Similar to Chl *a*, concentrations of DON were high (>8 μ mol L⁻¹) near the river mouth where they showed a vertically well-mixed pattern (Fig. 3i). However, a vertical gradient was observed for DON in the middle and outer plume, consistent with strong stratification in temperature and salinity. In this area, DON declined from >8 μ mol L⁻¹ in the upper layer to <5 μ mol L⁻¹ in the bottom layer. $\delta^{15}N_{DON}$ values exhibited a vertical gradient across the whole Changjiang plume transect (Fig. 3j), with values generally decreasing with water depth. Two low- $\delta^{15}N_{DON}$ patches were observed: one at the bottom of Y9b (-9.0‰), and the other in the middle layer at station Y5 (-7.0‰).

3.3. Changes in parameters with respect to salinity gradient

TSM sharply decreased from >2000 mg L⁻¹ at salinities <6 to ~200 mg L⁻¹ at salinities of ~23 (Fig. 4a). In Fig. 4, we divided the dataset

Fig. 2. Surface distribution of (a) Temperature-CTD, (b) Salinity-CTD, (c) TSM, (d) Chl a, (e) NO₃⁻, (f) $\delta^{15}N_{NO3}$, (g) PN, (h) $\delta^{15}N_{PN}$, (i) DON, and (j) $\delta^{15}N_{DON}$. The red bold line in (c) to (j) show the location of the turbidity front, with a TSM value of 5 mg L⁻¹. The smaller area covers of TSM, $\delta^{15}N_{NO3}$, DON and $\delta^{15}N_{DON}$ were resulted from the extremely low and/or undetectable values (Fig. 1). Note that the basic parameters, including temperature, salinity, TSM, Chl a, NO₃⁻, and $\delta^{15}N_{NO3}$ have previously been published but used here for contextual data (Hsiao et al., 2014; Tseng et al., 2014; Yan et al., 2017).



Fig. 3. Vertical distribution of (a) Temperature-CTD, (b) Salinity-CTD, (c) TSM, (d) Chl a, (e) NO₃⁻, (f) δ ¹⁵N_{NO3}, (g) PN, (h) δ ¹⁵N_{PN}, (i) DON and (j) δ ¹⁵N_{DON}.

into three zones according to certain criteria (see Section 4.1 and Fig. S2) to better reveal the dynamics of the three N species. At stations with salinities >23, TSM exhibited large variations ranging from 1.6 mg L⁻¹ to 171 mg L⁻¹. Compared to TSM, Chl *a* showed two different patterns along the salinity gradient separated by a salinity of 23 (Fig. 4b). The first pattern showed Chl *a* concentrations declining from 20.9 μ g L⁻¹ to 3.2 μ g L⁻¹ as the salinity increased from 2.2 to <23, while the second showed a much steeper slope, declining from 72.4 μ g L⁻¹ to <1.0 μ g L⁻¹ while the salinity increased from 24.0 to ~34.0.

Briefly, NO₃⁻ concentrations decreased as salinity increased (Fig. 4c). In regions with salinities <23, NO₃⁻ showed the best fit for conservative mixing behavior, and $\delta^{15}N_{NO3}$ varied in a small range between 5.8% and 6.5% (Fig. 4d). However, when salinity was >23, visible removal was observed in NO₃⁻. Correspondingly, the $\delta^{15}N_{NO3}$ values increased and reached as high as 15.9‰, accompanied by high Chl *a* concentrations. At stations with salinities >33, the variability of both NO₃⁻ and $\delta^{15}N_{NO3}$ was limited.

DON concentrations exhibited a similar decreasing trend to NO₃⁻ along the salinity gradient but with smaller variations (Fig. 4e). Higher concentrations of DON (27.1–32.7 µmol L⁻¹) were found in the lowsalinity waters. In regions with salinities >23, DON concentrations ranges slightly declined from 9.5–15.8 µmol L⁻¹ to 2.1–6.0 µmol L⁻¹. $\delta^{15}N_{DON}$ values ranged from 3.7% to 5.6% in regions with salinities <23 (Fig. 4f); however, at stations with salinities >23, $\delta^{15}N_{DON}$ values showed a wider range between -9.0% and 3.6%.

PN concentrations showed a similar pattern to those of TSM (Fig. 4a and g). PN was highest and reached up to $86.8-140.8 \ \mu mol \ L^{-1}$ at low-salinity waters, declined to $34.1-39.1 \ \mu mol \ L^{-1}$ when salinity varied between 11.4 and 17.0, and subsequently decreased to $18.6-19.4 \ \mu mol \ L^{-1}$

Fig. 4. Variations in (a) TSM, (b) Chl *a*, (c) NO₃⁻, (d) δ^{15} N_{NO3}, (e) DON, (f) δ^{15} N_{DON}, (g) PN, (h) δ^{15} N_{PN} along the salinity gradient (all data). The gray dashed lines represent a salinity of 23, dividing the entire dataset into two groups. The three datapoint colors represent samples from our defined zones (yellow for Zone 1, green for Zone 2, and mauve for Zone 3, defined in Section 4.1).



at a salinity of 23. When salinity increase above 23, PN sharply declined and varied over a relatively large range of 0.5–15.5 μ mol L $^{-1}$. Correspondingly, $\delta^{15}N_{PN}$ slightly increased from 0.2‰ to 2.3‰ before the salinities reached 17, and decreased slightly as the salinity increased to 23 (Fig. 4h). However, when salinities were >23, $\delta^{15}N_{PN}$ increased from -2.9% to 5.0‰ along with salinity gradient. The values of $\delta^{15}N_{PN}$ were slightly higher than $\delta^{15}N_{DON}$ but significantly lower than $\delta^{15}N_{NO3}$ where salinity is above 23.

4. Discussion

4.1. Relative proportions of the three N species in the total N pool

For convenience in the discussion, we classify the Changjiang plume into three zones. The zoning criteria are defined below and also shown in Fig. S2. Zone 1 is characterized by water samples with TSM concentrations >5.0 mg L⁻¹ throughout the entire water column. Zone 2 features by high Chl *a* concentrations >2 µg L⁻¹, including both samples taken from the surface layer (3-5 m) and those collected at depths of $\leq 20 \text{ m}$ (5 m < depth $\leq 20 \text{ m}$). The remaining samples (generally collected at the bottom in the outer plume) were classed as Zone 3.

The total N (TN) decreased as salinity increased in all three zones (Fig. 5a). To clarify the relationships among PN, DON and NO₃⁻, we compared their relative proportions in the TN pool and their isotopic compositions in the three zones (Fig. 5). The TN pool was dominated by DIN, primarily in the form of NO₃⁻ (Yan et al., 2017). DIN contributed 26.7–84.8% of the TN pool, with mean value of $66.1 \pm 10.8\%$ (n = 14), $62.0 \pm 14.5\%$ (n = 15) and $69.1 \pm 8.5\%$ (n = 27) in Zones 1, 2 and 3, respectively. However, the relative proportions of PN and DON in the TN pool were more variable. In Zone 1, where TN concentrations were higher than 165.3 µmol L⁻¹ (Fig. 5a), PN accounted for 20.6–46.7% of the TN pool while the percentage of DON was <16.4%. In waters with relatively lower TN (28.6–44.0 µmol L⁻¹) in Zone 1, the proportion of PN declined to 2.6–32.3% and DON increased to 13.8–21.1%. On average, the proportion of PN in the TN pool was 17.3 ± 13.6%, comparable to that of DON (16.6 ± 3.7%) in Zone 1. Compared with Zone 1, the



Fig. 5. (a) relative proportions of the three N species in the total N pool, and (b) their δ¹⁵N variations in the three defined zones (vertical solid grayed lines) along the Changjiang plume. The horizontal gray dashed line in (b) denotes an δ¹⁵N value of 0‰.

proportion of DON in Zone 2 was higher, ranging from 17.0–66.1% with an average contribution of 33.1 \pm 13.4%. PN only accounted for 0.8–15.6% of the TN pool, with a mean of 4.9 \pm 4.3%. In Zone 3, the mean proportions of PN and DON were 5.4 \pm 3.6% and 25.6 \pm 8.8%, respectively. Overall, the relative contributions of these three N species to the TN pool in the Changjiang plume was generally DIN > DON>PN. This is consistent with observations in the subtropical eutrophic Pearl River estuary (Ye et al., 2018; Ye et al., 2016), but differs from those reported from a near-pristine estuary (the Noosa River estuary) and coastal areas (e.g., Scottish fjords), which had proportions in the order of DON>DIN > PN (Moschonas et al., 2017; Wells and Eyre, 2018).

NO₃⁻ had the highest δ^{15} N values of the three species (4.9–15.9%; Fig. 5b), with a mean δ^{15} N_{NO3} value of 5.5 ± 2.2‰ (n = 67). δ^{15} N_{DON} values generally ranged between -9.0% and 5.6‰ (Fig. 5b), which are lighter than δ^{15} N in bulk-DON and high molecular weight DON (HMW-DON) in the oligotrophic ocean (Knapp et al., 2012; Meador et al., 2007). However, they are similar to δ^{15} N_{DON} values reported from the subtropical estuaries (-8.4% to 5.0‰) (Wells and Eyre, 2018; Ye et al., 2018) and the δ^{15} N of amino acids (-18% to 0‰) generated from freshly-produced algal HMW-DON (Calleja et al., 2013). Generally, values of δ^{15} N_{DON} (-2.9% to 5.0‰, 2.0 ± 1.6‰, n = 101) were slightly higher than δ^{15} N_{DON} (-9.0% to 5.6‰, $-0.1 \pm 2.7\%$, n = 58). Similarly, more depleted δ^{15} N in DON than PN has been observed in estuaries worldwide, suggesting a tight coupling of DON production and consumption (Wells and Eyre, 2018; Ye et al., 2018).

In Zone 1, which had high turbidity, $\delta^{15}N_{NO3}$ values were relatively consistent (5.8 \pm 0.4‰), which mainly due to the co-occurrence of water-column nitrification and sedimentary denitrification limiting changes in $\delta^{15}N_{NO3}$ (Yan et al., 2017). However, more variability was observed in $\delta^{15} N_{PN}$ (-1.7‰ to 4.6‰, 1.8 \pm 1.6‰ on average) and δ^{15} N_{DON} (-3.6% to 5.6%, 0.7 \pm 3.0% in mean), indicating the more complicated dynamics of organic N due to the co-influence of terrestrial inputs and internal cycling (Wells and Eyre, 2018; Ye et al., 2018). In Zone 2, the mean $\delta^{15}N_{NO3}$ value (8.4 \pm 3.1‰) was higher than $\delta^{15}N_{PN}$ (0.8 \pm 1.8%). Low $\delta^{15}N_{PN}$ values corresponded to high $\delta^{15}N_{NO3}$ accompanied by high Chl a concentrations and moderate concentrations of NO_3^- (Fig. S3), suggesting that phytoplankton preferentially consume isotopically light NO_3^- to produce PN, leaving more ¹⁵N remaining in the residual NO_3^- pool (Yan et al., 2017). In Zone 3, $\delta^{15}N_{NO3}$ showed a similar distribution (Fig. 5b) and a close average value (5.5 \pm 0.6%) to Zone 1. The $\delta^{15}N_{NO3}$ value at the bottom of station Y4 (5.0%, the NKBC end-member) was close to that of typical deep seawater (Casciotti, 2016; Kawagucci et al., 2018), showing the influence of the NKBC in this zone (Liu and Kaplan, 1989; Yan et al., 2017). However, the mean δ^{15} N value was higher in PN (2.7 \pm 0.7‰) than DON (-4.0 ± 3.0 ‰). A similar trend of a wider range but lower values of $\delta^{15}N_{DON}$ relative to $\delta^{15}N_{PN}$ has also been observed in the Brisbane River estuary during the dry season (Wells and Eyre, 2018) and in the Elbe River estuary throughout the year (Schlarbaum et al., 2010), which may indicate significant alteration of the DON pool by internal cycling in addition to the influence of soil-derived organic matter inputs that are more depleted in $\delta^{15}N_{DON}$ (Wells and Eyre, 2018; Ye et al., 2018). However, this is contrary to the traditional framework for oligotrophic surface ocean that the breakdown of DON to NH₄⁺ has a greater isotope effect than the production of DON from PN, leading to depleted $\delta^{15}N_{PN}$ but enriched $\delta^{15}N_{DON}$ (Knapp et al., 2011; Sigman and Fripiat, 2018; Thibodeau et al., 2017). Nevertheless, the dynamic nature of N in this region, including multiple transformations, has resulted in the differentiation of isotopic characteristics within the inorganic and organic N pools.

4.2. Nonconservative behaviors and low δ^{15} N values of DON

In most estuaries investigated, dissolved organic carbon displays a conservative behavior (Mantoura and Woodward, 1983; Millero,

2013); however, DON may not be conservative (Kim et al., 2020; Schlarbaum et al., 2010; Ye et al., 2018). Although field studies of DON behaviors/dynamics insight from δ^{15} N remain insufficient, both DON uptake rates and bioavailability studies showed that DON could be utilized as N source for phytoplankton growth (Glibert et al., 2004; Moschonas et al., 2017; Zhang et al., 2015), while some studies showed DON adsorption may occur in turbid water (Berman and Bronk, 2003; Schlarbaum et al., 2010; Ye et al., 2018). These processes may alter the concentrations and isotopic compositions of DON in estuary at the same time.

To better understand the dynamics of DON, we first attempted to separate the influences of biological activity from physical mixing using a conservative three end-member model, which has been successfully applied to study the dynamics of NO₃⁻ and particulate organic carbon (POC) in this study area (Wang et al., 2016; Yan et al., 2017). Selection of the three end-members (CJ, ECSSW, NKBC) followed Yan et al. (2017) and are based on the T-S diagram. Values of DON and $\delta^{15}N_{DON}$ adopted in the three end-member mixing model are summarized in Table 1. Details on the calculation methods can be found in the supplementary materials (Text S1).

DON concentrations mostly fell below the conservative mixing area of the three end-members (Fig. 6a), indicating a removal of DON. This is also supported by a scatter plot of $\delta^{15}N_{DON}$ against the inverse of DON concentrations (Fig. 6b), where very few data points fell within the physical mixing area. A similar phenomenon has been observed in Eurasian coastal Arctic ecosystems (Thibodeau et al., 2017), suggesting active DON removal and subsequent isotope effects can occur in river plumes globally. To better reveal the DON dynamics and identify the main processes involved, we plotted the $\delta^{15}N_{DON}$ -offsets versus the DON-offsets (the offsets are the deviations between theoretical and measured values; Fig. 6c). We can see that DON deficits ($-20 \mu mol L^{-1}$ to $-0.5 \ \mu\text{mol} \ \text{L}^{-1}$) are accompanied by negative $\delta^{15}N_{\text{DON}}$ -offsets (-11.2% to -0.5%), with mean values of $-3.5 \pm 3.7 \ \mu mol \ L^{-1}$ and $-3.6 \pm 2.2\%$ (n = 43), respectively. These deviations have high confidence, supported by an analysis of the occurrence frequencies of DON-offsets and $\delta^{15}N_{DON}$ -offsets (Fig. S4).

Based on current knowledge, the best explanation for the observed DON removal and negative shift in $\delta^{15}N_{\text{DON}}$ values is the selective adsorption of ^{15}N enriched moieties onto particulate matter in Zones 1 and 3 where TSM was high, while phytoplankton uptake of DON, producing a negative isotope effect, predominated in Zone 2 where Chl *a* was high.

Zone 1 was located near the river mouth where turbidity was highest (Figs. 2c and 3c), while Zone 3 was at the bottom of the offshore Changjiang plume where TSM was >5 mg L⁻¹ (Fig. 3c). High levels of TSM in these two areas provide greater potential for the selective adsorption of ¹⁵N enriched moieties onto particulate surfaces (Berman and Bronk, 2003; Schlarbaum et al., 2010; Schlarbaum et al., 2011; Ye et al., 2018), and thus result in the observed DON loss and negative shift in $\delta^{15}N_{DON}$ in Zone 1 ($-4.9 \pm 5.7 \mu$ mol L⁻¹ and $-3.7 \pm 1.6\%$) and Zone 3 ($-2.1 \pm 2.0 \mu$ mol L⁻¹ and $-3.0 \pm 2.6\%$). The observed low DON ($<5 \mu$ mol L⁻¹) and $\delta^{15}N_{DON}$ (<-1%) accompanied by high PN ($>1 \mu$ mol L⁻¹) and enriched $\delta^{15}N_{PN}$ (>3%) at the bottom of Zones 1 and 3 are also evidence for the active selective adsorption of enriched ¹⁵N from DON onto PN, similar with previous observations in the Elbe estuary (Schlarbaum et al., 2010; Schlarbaum et al., 2011)

Table 1	
The end-member values adopted in the three end-member mixing	g model.

End-member	S	Potential Temperature (°C)	DON (μ mol L ⁻¹)	δ ¹⁵ N _{DON} (‰)
CJ	2.2	29.4	32.7	5.2
ECSSW	33.4	29.2	5.2	3.5
NKBC	34.4	19.7	6.0	1.2

CJ, ECSSW and NKBC represent the Changjiang, East China Sea Surface Water and the Nearshore Kuroshio Branch Current, respectively.



Fig. 6. Scatter plot of (a) DON versus salinity, (b) $\delta^{15}N_{DON}$ versus the inverse DON concentrations, (c) $\delta^{15}N_{DON}$ -offsets versus DON-offsets. The black squares in (a) and (b) show the three end-members (CJ, ECSSW and NKBC) values, while the black lines indicate mixing among the end-members. The gray shadows in (c) show the uncertainties of $\pm 0.5 \text{ µmol L}^{-1}$ for the DON-offsets and $\pm 1.1\%$ (the isotope bias from NH^{\pm}) for the $\delta^{15}N_{DON}$ -offsets, respectively. The yellow, green and pink dashed lines are the best-fit modeled isotope effect for Zone 1 ($^{15}\epsilon \approx -8\%$ to -20%), Zone 2 ($^{15}\epsilon \approx -40\%$) and Zone 3 ($^{15}\epsilon \approx -5\%$ to -20%), respectively.

and the Pearl River estuary (Ye et al., 2018). This suggests that active interactions had occurred between the DON and PN pools in the Changjiang plume. Alternatively, the salinity-mediated release of NH_4^+ from dissolved organic matter (DOM) is another mechanism to explain the DON deficit, since NH_4^+ adsorbed to humic material can be released into the surrounding water when transported into more saline waters (See and Bronk, 2005; Sipler and Bronk, 2015). This may occur in Zone 1 which had a large salinity gradient (Fig. 4) and also relatively high values of NH_4^+ near the river mouth (Hsiao et al., 2014). However, it was not possible to determine the magnitude of δ^{15} N during this process, but we cannot rule out its influence in Zone 1. In Zone 3 which was influenced by Kuroshio water, N₂ fixation might partly contribute to the negative shift in $\delta^{15}N_{DON}$ since high N₂ fixation rates had been observed in the upstream Kuroshio and the Kuroshio bifurcation region of the ECS (Chen et al., 2008; Shiozaki et al., 2015) during summer. Thus, we may argue that the intrusion of the Kuroshio into Zone 3 could be a direct source of δ^{15} N-depleted DON produced by diazotrophs (Knapp et al., 2016; Meador et al., 2007). Although the release of bioavailable N (such as NH_4^+ and dissolved primary amines) from DOM by photochemical reactions could be an important sink for DON in seawater and brackish water environments (Bronk et al., 2010), its contribution seems to be limited by light in Zone 1 where the turbidity was high (Fig. 2c) and in Zone 3 where water depth was deep (Fig. 3c). Similarly, phytoplankton uptake of DON could also be ruled out due to the light limitation. Moreover, heterotrophic bacterial degradation/ammonification can also be excluded since it will breakdown DON with an isotope effect of ~5% (Sigman and Fripiat, 2018), thus enriching $\delta^{15}N_{DON}$ after bacterial alteration (Calleja et al., 2013) rather than leading to a negative shift in $\delta^{15}N_{DON}$ as we observed (Fig. 6c).

In Zone 2, where Chl a was high (Fig. 4b), DON uptake by phytoplankton is an important DON sink (Sipler and Bronk, 2015). Although significant NO₃⁻ uptake by phytoplankton has been reported in Zone 2 (Yan et al., 2017), the mean drawdown of DON ($-3.0 \pm 2.3 \ \mu mol \ L^{-1}$, n = 9) could have contributed up to 16 \pm 12% of the TDN drawdown, with the remaining supporting by NO_3^- assimilation. The phenomenon of synchronous consumption of DON and NO₃⁻ has been observed in Loch Creran, a near-pristine coastal area in the Scottish fjords (Moschonas et al., 2017), which likely attributed to the taxon-specific differences in affinity for multiple N substrates (Fawcett et al., 2011; Moschonas et al., 2017). For example, diatoms generally prefer high nutrient concentrations (Xiao et al., 2017), while some phytoplankton including dinoflagellates and chlorophytes are less sensitive to nutrient concentrations (Xiao et al., 2017) and can also take up low-molecular weight DON like urea, dipeptides and some amino acids via pinocytosis (Bronk, 2002; Glibert and Legrand, 2006; Sipler and Bronk, 2015). Thus, synchronous consumption of DON and NO_3^- can also occur in the Changjiang plume since, in addition to diatoms, other taxon such as dinoflagellates, cyanobacteria, and prochlorophytes are also present (Furuya et al., 2003; Liu et al., 2016b). However, we would expect that $\delta^{15}N_{DON}$ would become enriched, as DON being used results in an isotope effect of around 5-6‰ (Thibodeau et al., 2017; Zhang et al., 2020), which is inconsistent with the observed negative shift in $\delta^{15}N_{DON}$ (Fig. 6c). Alternatively, the newly-fixed N produced by diazotrophs, which can be stimulated by the river plume due to the availability of nutrients (Voss et al., 2006; Weber et al., 2016), could be transferred rapidly through the DON pool (Caffin et al., 2018; Knapp et al., 2016), which would decrease $\delta^{15}N_{\text{DON}}$ (-0.7% to -0.3%) (Meador et al., 2007) but increase DON. We believe that N₂ fixation can explain these datapoints (Fig. 6c) with slight DON addition (+0.6 μ mol L⁻¹ to +1.1 μ mol L⁻¹) and negative shift in $\delta^{15}N_{DON}$ (-1.1% to -4.1%), although other studies in the oligotrophic ocean showed that diazotroph fixed N was transferred rapidly to bacteria, non-diazotrophic phytoplankton, and zooplankton communities rather than released to the ambient waters when unicellular cyanobacteria group B (UCYN-B) dominated (Caffin et al., 2018; Meador et al., 2007). Yet, N₂ fixation cannot explain those datapoints showing negative shift in $\delta^{15}N_{DON}$ (-3.3% to -8.7%) with significant DON deficit $(-0.6 \,\mu\text{mol L}^{-1} \text{ to } -7.5 \,\mu\text{mol L}^{-1})$. This phenomenon can only be explained by DON removal processes, such as decomposition/remineralization and photolysis/photo-ammonification. However, recent studies showed that the breakdown of DON (i.e., decomposition or

remineralization) may elevate $\delta^{15}N_{DON}$ after bacterial alteration in general (Calleja et al., 2013; Sigman and Fripiat, 2018). This is consistent with the observation that the coupling effects of photo-ammonification and decomposition can yield a positive isotope effect of 4.2–5.6‰ in the Arctic ecosystem (Thibodeau et al., 2017). Therefore, the influence of DON remineralization and photo-ammonification should be limited. Importantly, since the labile DON uptake by phytoplankton was reported during the succession from a diatom bloom to dinoflagellate bloom in the Changjiang estuary and its adjacent shelf (Zhang et al., 2015), where the decrease in both DON concentration and bioavailability were observed. Therefore, we suggest that the most likely cause for simultaneous reduction in DON and $\delta^{15}N_{DON}$ is the DON uptake by phytoplankton with a strong inverse isotope effect in Zone 2.

Based on these thorough discussions about the internal processes in the DON pool in this region, an inverse isotope effect is required to induce the negative shift in $\delta^{15}N_{\text{DON}}$ as the overall concentration of DON decreases. To further evaluate the isotope effect during DON consumption ($^{15}\varepsilon$ -DON), we applied a best-fit model following the Rayleigh model for DON in the three zones (Sigman et al., 1999; Treibergs and Granger, 2017). Datapoints inside the mixing field were averaged separately for the three zones as the initial DON available for consumption. We found that the best-fit ${}^{15}\varepsilon$ -DON was in a range of -8% to -20%for Zone 1, -40% for Zone 2, and -5% to -20% for Zone 3 (Fig. 6b). The differences in derived $^{15}\varepsilon$ -DON values among the three zones were caused by the different DON removal pathways present (Thibodeau et al., 2017) as well as the environmental conditions (e.g. turbidity, light intensity, salinity, substrate concentrations) to which they have been exposed (Treibergs and Granger, 2017). Although such inverse values of ¹⁵ε-DON are different from those of DON removal processes occurring in coastal Arctic ecosystems and the oligotrophic ocean (Treibergs and Granger, 2017; Zhang et al., 2020), they are consistent with the selective adsorption of ¹⁵N enriched moieties onto particulate surfaces in the estuary (Schlarbaum et al., 2010; Schlarbaum et al., 2011).

Therefore, the best explanation for the low δ^{15} N of DON and its nonconservative behaviors is the selective adsorption of ¹⁵N enriched moieties of DON onto particulate surfaces Zones 1 and 3, with overall ¹⁵ ε -DON ranges of -8% to -20%, and -5% to -20%, respectively, and primarily phytoplankton uptake of DON in Zone 2, accompanied by an inverse ¹⁵ ε -DON of -40%. These results show that DON is reactive and its nonconservative behaviors and dynamics differ in the Changjiang plume from those in other coastal regions.

4.3. PN newly produced by phytoplankton uptake

Interestingly, completely different patterns were found for 3 zones in the scatter plots of PN and $\delta^{15}N_{PN}$ versus Chl *a* (Fig. 7a and b). Zone 1 was characterized by high concentrations of PN but low values of both Chl *a* concentrations and $\delta^{15}N_{PN}$, implying that PN was not directly produced by in situ phytoplankton. This is a reasonable finding since relatively high amounts of Chl a have been confirmed to be allochthonous from the upper river (Tseng et al., 2014). Thus, PN may primarily originate from terrestrial inputs since $\delta^{15}N_{PN}$ generally fell in a narrow range (1.5-3.0‰) in the inner plume (belong to Zone 1) where TSM was >42 mg L⁻¹ (Fig. 7c), which was also reflected by $\delta^{13}C_{POC}$ values in previous studies (Gao et al., 2014; Kao et al., 2003). In Zone 3 Chl a was lowest, while PN was typically <5 $\mu mol \ L^{-1}$ and $\delta^{15} N_{PN}$ ranged between -0.2% and 5.0‰. Such low PN and variable $\delta^{15}N_{PN}$ can primarily be attributed to the remineralization of particulate organic material, supporting by the observed additions of NO₃⁻ and the slightly negative shift in $\delta^{15}N_{NO3}$ but positive shift in $\delta^{18}O_{NO3}$ (Yan et al., 2017). Compared with Zone 1, Zone 2 featured with high levels of Chl *a* but low PN and variable $\delta^{15}N_{PN}$, suggesting that the properties of PN might vary with changes in environmental conditions (Gao et al., 2014; Zhang et al., 2007). Although no obvious relationship was found between $\delta^{15}N_{PN}$ and Chl *a* when Chl *a* concentrations were < 21 µg L⁻¹, $\delta^{15}N_{PN}$ positively correlated with Chl *a* (slope = 0.03 ± 0.02 , r = 0.75, p = 0.14) when Chl a was >21 µg L⁻¹, implying the production of PN by phytoplankton. This is supported by the large variability in $\delta^{15}N_{PN}$ and also the co-enrichment of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ (Yan et al., 2017), both which may have been induced by the intense phytoplankton growth as TSM decreased in the outer surface plume (Fig. 7c) and affected the features of PN synchronously (Casciotti et al., 2008; Sigman and Fripiat, 2018). Moreover, in these high-Chl *a* regions, PN was characterized by light $\delta^{15}N$ (-0.8% to -2.9%), which may be associated with the isotope effect during phytoplankton uptake of NO_3^- and DON to form PN (Liu et al., 2007; Sigman and Fripiat, 2018). This is consistent with evidence from the linear regression of POC versus PN (6.3 \pm 0.1, r = 0.9980, p < 0.0001, Fig. 7f) which is close to the Redfield ratio of 6.6, suggesting that the particulate organic matter (POM) originated from marine phytoplankton (Wu et al., 2016).

To simulate the isotope effect during the production of PN via $NO_3^$ uptake ($^{15}\varepsilon$ -NO₃⁻), a scatter plot of $\delta^{15}N_{NO3}$ versus $\delta^{15}N_{PN}$ was made (Fig. 7e). In Zone 2 we found that $\delta^{15}N_{NO3}$ correlates well with $\delta^{15}N_{PN}$ and the differences between them mostly fell in the range of 8–13‰, which can be defined as the isotope effect (Finlay and Kendall, 2007; Tamše et al., 2014). This is consistent with estimates from field studies in coastal areas due to the uptake of local NO_3^- (Tamše et al., 2014). However, it is slightly higher than the estimated isotope effect (5-10%) based on dual isotopic compositions of NO₃⁻ during the same cruise (Yan et al., 2017), which may be the result of some production of PN from DON uptake by phytoplankton (16 \pm 12%) with an inverse isotope effect of around -40%, as previously mentioned in the discussion (Section 4.2). We further confirmed this by examining the differences between $\delta^{15}N_{NO3+DON}$ and $\delta^{15}N_{PN}$ (Fig. 7f), which showed that the isotope effect decreased to within 4-8‰ when we consider both the influence of DON and NO_3^- . Differing from Zone 2, there were no correlations between $\delta^{15}N_{NO3}$ (which had little variability) and $\delta^{15}N_{PN}$ in both Zones 1 and 3, suggesting that PN has been modified and/or altered by biological processes. When we consider the influence of DON, the datapoints showed a discrete trend in the scatter plot of $\delta^{15}N_{NO3}$ $_{+DON}$ versus $\delta^{15}N_{PN}$ (Fig. 7f), especially in Zones 1 and 3, also highlighting the bioactivity of DON and its influence on PN.

To further identify the sources of PN, we combined our dataset with previously reported values for $\delta^{13}C_{POC}$ and molar C/N ratios (Fig. 7g and h; Wang et al., 2016), since the geochemical characteristics may reflect the origin of organic matter in the study area (Liu et al., 2007). The geochemical characteristics of three potential end-members (namely riverine, marine and deltaic sediments) are shown in Fig. 7g and h, and also listed in Table 2 for reference.

Comparing with historical datasets in the same study area (Gao et al., 2008; Kao et al., 2003; Zhang et al., 2007), values of $\delta^{15}N_{PN}$ were lighter but $\delta^{13}C_{POC}$ was heavier in this study (Fig. 7g), yet, consistent with recent study (Gao et al., 2014). Such relatively low values of $\delta^{15}N_{PN}$ observed in the Changjiang plume were lower than those in the Scheldt estuary (0-25%; Middelburg and Herman, 2007; Middelburg and Nieuwenhuize, 1998) and the Elbe estuary (5–15‰; Middelburg and Herman, 2007), but similar to those in the Danshuei estuary (-16.4% to 3.8%; Liu et al., 2007). This may be attributed to the differences in the dominant N forms (such as NH_4^+ or NO_3^-), isotope effects, phytoplankton activity, and water residence times (Liu et al., 2013; Middelburg and Herman, 2007; Zhang et al., 2007). For example, values of $\delta^{15}N_{PN}$ can be elevated when PN is from ^{15}N enriched N sources, such as residual NH₄⁺ accompanied by intensive algal uptake (Middelburg and Herman, 2007; Middelburg and Nieuwenhuize, 1998). However, isotopically light δ^{15} N in PN can be found when PN is produced during uptake of fresh riverine NH⁺, due to the maximum isotope effect (~20‰) at NH₄⁺ concentrations of ~100 μ mol L⁻¹ (Liu et al., 2013). Additionally, PN originating from phytoplankton incorporating ¹⁵N-depleted NO₃⁻ is also likely have depleted $\delta^{15}N_{PN}$ values,



which generally occurs when NH_4^+ inhibition of NO_3^- uptake ceases (Liu et al., 2007). Our results support NO_3^- uptake since an obvious removal of NO_3^- accompanied by ¹⁵N-enriched NO_3^- and relatively depleted $\delta^{15}N_{PN}$ were observed in Zone 2 (Fig. 3f and h). However, in our case, the incorporation of ¹⁵N-depleted NO₃⁻ by phytoplankton in Zone 2 was stimulated by low turbidity rather than by the lessening of NH₄⁺ inhibition, since the background of NH₄⁺ was generally low across the whole Changjiang plume. Moreover, phytoplankton uptake of NO₃⁻ can only partially explain the observed ¹⁵N-depleted PN in Zone 2 since the differences between $\delta^{15}N_{NO3}$ and $\delta^{15}N_{PN}$ ranged from 3.0% to 13.0% (Fig. S3), which is higher than the ${}^{15}\varepsilon$ -NO₃ (5–10%) estimated from the dual isotopic compositions of NO₃⁻ (Yan et al., 2017). As previously mentioned, the $\delta^{15}N_{PN}$ depletion in Zone 2 can partly be attributed to DON uptake by phytoplankton, which can produce higher δ^{15} N enrichment in PN than in DON. However, in Zones 1 and 3, the selective adsorption of δ^{15} N enriched moieties onto particulate surfaces is likely the dominant process controlling the isotopically light PN. Therefore, our results suggest a tight coupling of PN with other N pools (NO_3^- and DON).

Based on the above analysis, we found that the origin of organic matters cannot always be traced through its geochemical characteristics, such as the isotopic compositions of POC and PN, and C/N ratios (Fig. 7g and h), since they can be modified due to intensive bioactivities (Middelburg and Herman, 2007), similar to what we observed in the Changjiang plume. From the scatter plots of $\delta^{15}N_{PN}$ versus $\delta^{13}C_{POC}$ (Fig. 7g), we found that the data points are mostly located near the deltaic and marine sources rather than riverine inputs, implying a limited influence of terrigenous material. However, near the river mouth where TSM was high, the relatively small variations of $\delta^{15}N_{PN}$ likely resulted from the inputs of terrestrial material (Fig. 7c). By adding molar C/N ratios for comparison, $\delta^{15}N_{PN}$ showed high similarity to marine sources, yet still influence by the isotope effect (Fig. 7h). This differs from the historical data, which suggests that PN was influenced by the mixing of riverine inputs and marine sources (Zhang et al., 2007). Such discrepancy may result from the variability of molar C/N ratios, which ranged from 4.7 to 6.7 with mean value of 7.2 \pm 0.0 for Zone 2, 6.3 \pm 0.1 for Zone 2, and 5.7 \pm 0.1 for Zone 3 (Fig. 7d). These are close to the C/N ratios of 4-7 for POM in the same area (Zhao and Gao, 2019) and the southern ECS (Liu et al., 2018), but lower than the C/N ratios of 7–15 in the earlier studies (Gao et al., 2008; Gao et al., 2014; Kao et al., 2003; Zhou et al., 2006). On the one hand, it may be associated with the degree of degradation of organic material, which will increase C/N ratios due to the preferential decomposition of N-rich organic material (Savoye et al., 2003). However, as previously mentioned, PN in Zone 2 was newly produced by phytoplankton uptake and thus was characterized by relatively low C/N ratios, consistent with the observed C/N ratios of phytoplankton in Salem Harbor (Hubeny et al., 2017). On the other hand, high amounts of DIN adsorbed onto the organic material may also account for the low molar C/N ratios. This can occur in the Changjiang plume, especially in Zone 1 where turbidity was high and NH_4^+ was ~1.2 µmol L⁻¹ (Hsiao et al., 2014), providing more opportunities for NH_4^+ adsorption onto the organic matter. Alternatively, to some extent the levels of turbidity can reflect the contribution of terrigenous material since riverine inputs are generally the main source of TSM in coastal areas. In this study, TSM was mostly <100 mg L⁻¹, whereas it was previously reported to be 100–1000 mg L⁻¹ due to sediment transport and resuspension (Zhang et al., 2007). In this regard, we believed that marine-derived PN was dominant in the Changjiang plume, yet significantly influenced by intensive biological processes involving DON transformations.

5. Conclusion

This study documents the horizontal and vertical distribution of PN and DON concentrations and isotopic compositions in the Changjiang plume. Although both PN and DON decreased seaward, and $\delta^{15}N_{PN}$ also generally increased moving offshore, $\delta^{15}N_{DON}$ showed the opposite trend. DON and PN represented 25.6 \pm 12.1% and 8.1 \pm 9.1% of the TN pool, respectively. For the entire Changjiang plume, the mean values of δ^{15} N were lowest for DON, slightly higher for PN, and highest for NO₃, implying active biological transformations have occurred among the three N pools. Combining the geochemical characteristics of DON (concentration and isotopic composition) with a conservative mixing model, we found that the observed datapoints deviated from the mixing areas, suggesting nonconservative DON behaviors. The negative shifts in $\delta^{15}N_{\text{DON}}$ accompanied by obvious DON deficits across the entire Changjiang plume confirms that DON was active and can be modified by biological processes. In the offshore surface plume where TSM was low and thus supported good conditions for the growth of phytoplankton, the negative offsets in both DON and $\delta^{15}N_{DON}$ were likely driven by the utilization of DON by phytoplankton (with an isotope effect of -40%), accounting for $16 \pm 12\%$ of the PN production. This information is vital to the management of algal blooms in estuaries and other coastal areas. However, near the river mouth and at the bottom of the offshore plume where TSM was high, the nonconservative behaviors of DON were mainly controlled by the selective adsorption of ¹⁵N enriched moieties onto particulate surfaces, with an isotope effect of approximately -5% to -20%. Our results show active transformations among the three N pools, with DON playing an important role in shaping the isotopic characteristics of PN in the Changjiang plume. In other words, we cannot trace the sources of PN solely through the biogeochemical characteristics of organic matter ($\delta^{15}N_{PN}$, $\delta^{13}C_{POC}$ and the molecular C/N ratios) since they can be modified during the transport of water in the Changjiang plume. Further studies are urgently needed to unveil the transformation mechanisms among the PN, DON and NO₃⁻ pools in the changing coastal regions since the relative contributions of the specific processes may vary yearly. Applications of Fourier Transform Ion Cyclotron Resonance Mass Spectrometry may help to constrain the specific processes at the molecular level.

CRediT authorship contribution statement

Shuh-Ji Kao and Minhan Dai organized the project. Jin-Yu Terence Yang collected the samples. Xiuli Yan and Hongjie Wang carried out chemical analyses. Xiuli Yan, Shuh-Ji Kao, Jin-Yu Terence Yang, Hongjie Wang, Min Nina Xu, Minhan Dai conducted data analysis and wrote the manuscript. All authors were involved in discussions.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Fig. 7. Scatter plots of (a) PN versus Chl *a* (b) $\delta^{15}N_{PN}$ versus Chl *a*, (c) $\delta^{15}N_{PN}$ versus TSM, (d) POC versus PN, (e) $\delta^{15}N_{NO3}$ versus $\delta^{15}N_{PN}$, (f) $\delta^{15}N_{NO3+DON}$ versus $\delta^{15}N_{PN}$, (g) $\delta^{15}N_{PN}$ versus Chl *a*, (c) $\delta^{15}N_{PN}$ versus TSM, (d) POC versus PN, (e) $\delta^{15}N_{NO3}$ versus $\delta^{15}N_{PN}$, (f) $\delta^{15}N_{NO3+DON}$ versus $\delta^{15}N_{PN}$, (g) $\delta^{15}N_{PN}$ versus molar C/N ratios. The solid lines in (e) and (f) are the isotope effects during the formation of PN via NO₃⁻⁻ uptake (8‰ and 13‰) and via co-assimilation of NO₃⁻⁻ and DON (4‰ and 9‰), respectively. In (g) and (h), gray symbols represent data from previous studies (Gao et al., 2008; Wu et al., 2003; Zhang et al., 2007), and the three end-members (riverine, marine and deltaic) are also obtained from previous studies (Gao et al., 2014; Kao et al., 2003; Wu et al., 2007; Zhou et al., 2006).

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Table 2

Characteristics of the three end-members of POM in the Changjiang plume.

End-member	Salinity	C/N (mol/mol)	δ ¹³ C (‰)	δ ¹⁵ N (‰)	References
Riverine Deltaic ^a	<0.4 /	$\begin{array}{c} 12.9 \pm 2.2 \\ 8.2 \pm 1.2 \end{array}$	-26.4 ± 0.7 -22.5 ± 0.4	$\begin{array}{l} 4.0 \pm 1.7 \\ 3.4 \pm 0.7 \end{array}$	Gao et al., 2014 Kao et al., 2003 Zhou et al., 2006 Gao et al., 2008
Marine	31.8 ± 2.5	7.2 ± 0.8	-20.5 ± 1.3	6.4 ± 1.1	Wu et al., 2007

^a The values for the deltaic end-member are averaged from the previous studies (Gao et al., 2008; Kao et al., 2003; Zhou et al., 2006) in the ECS.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.scitotenv.2021.151678.

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